Field Response of Mediterranean Fruit Fly (Diptera: Tephritidae) to Ceralure B1: Evaluations of enantiomeric B1 Ratios on Fly Captures

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ABSTRACT (−)-Ceralure B1 (ethyl-*cis*-5-iodo-*trans*-2-methylcyclohexane-1-carboxylate), a male attractant for the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is significantly more attractive than trimedlure (*tert*-butyl esters of 4(5)-chloro-2-methylcyclohexane-1-carboxylate), the current standard male attractant used in detection programs. This article reports studies that compare the effectiveness of racemic ceralure B1, mixtures of racemic ceralure B1 and pure (−)-ceralure B1, and trimedlure in field tests conducted in Hawaii, Africa, and Spain with wild Mediterranean fruit flies and in Florida with sterile released Mediterranean fruit fly. Trapping results showed that doses of (−)-ceralure B1 of 87.5 and 75% are just as effective as the 98% (−)-ceralure B1 and the racemic form to be almost as attractive. In nearly all studies, the racemic ceralure B1 was significantly better than trimedlure. These studies suggest that the racemic ceralure B1 could be a viable replacement for trimedlure in areawide detection programs for Mediterranean fruit fly. Synthesizing racemic ceralure B1 instead of a specific stereoselective enantiomer of ceralure B1 would likely be more cost-effective to produce and also might be useful in control as well as detection of this pest.

KEY WORDS *Ceratitis capitata*, lure, attractant, ceralure B1, trimedlure

THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wiedemann) is one of the most important pests of economic importance worldwide. This insect is known to attack >250 fruit and vegetable crops (Liquido et al. 1991) and is considered a serious quarantine pest. The threat of Mediterranean fruit fly establishment has always been a high priority for states/countries engaged in international trade due to the fly's quarantine importance, and an ongoing search for new and improved semiochemical-based control and detection methods remain a high priority. The current male attractant used for detection of the Mediterranean fruit fly is trimedlure. Trimedlure is a mixture of the *tert*-butyl esters of 4- and 5-chloro-(*E* and *Z*)-2-methylcyclohexane-1-carboxylic acids (Beroza et al. 1961), which is formulated in a 2-g polymeric plug for controlled release and prolonged evaporation (Leonhardt et al. 1987, 1989).

Ceralure, an iodinated analog of trimedlure, was developed by McGovern and Cunningham (1988) and found to be more persistent than trimedlure in field trials (Avery et al. 1994, Warthen et al. 1994, 1997; Leonhardt et al. 1996). Ceralure, like trimedlure, is composed of 16 regio- and stereoisomers, of which the B1 isomer was reported to be the most attractive (Warthen et al. 1994). Recently, specific stereoselective enantiomers of ceralure B1 have been synthesized and the (−)-enantiomer of ceralure B1 was shown to be more attractive than the (+)-enantiomer of ceralure B1 (Raw and Jang 2000, Jang et al. 2001). Additional studies also have shown that the (−)-enantiomer of ceralure B1 (ethyl *cis*-5-iodo-*trans*-2-methylcyclohexane-1-carboxylate) is ≈4–9 times more attractive to laboratory-released sterile and wild Mediterranean fruit flies than commercial trimedlure and ceralure (Jang et al. 2001, 2003).

In addition to their use in detection traps, some male lures have been successfully used in "male annihilation" mass trapping control programs. Semiochemical-based attractants for fruit flies are also one of the key tools used in integrated pest management (IPM) programs and for control and eradication of tephritid fruit fly pests. The male annihilation technique, which has been used successfully for some *Bactrocera* species of fruit flies (Steiner et al. 1965, Metcalf and Metcalf 1992), eliminates the majority of the male population and eventually reduces the population because the females are unable to find mates and reproduce. The success of this technique for population control relies

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on the effectiveness of the attractant used to trap the male flies.

An important aspect in determining the best attractant for use in control programs is not only its efficacy but also the cost of producing the product for commercial use. The (−)-enantiomer of ceralure B1, although a superior molecule for attracting Mediterranean fruit fly males may be prohibitively expensive to produce given the known existing synthesis route (Raw and Jang 2000). The racemic ceralure B1 is less difficult to synthesize, requires fewer steps to produce (Khrimian et al. 2003), and arguably less costly to manufacture.

In this study, we conducted research to determine if racemic (±) ceralure B1 or (−)-ceralure B1-biased ratios of the (±) mixture might be as effective as the pure (−)-enantiomer. Field tests were conducted in Hawaii, Africa, Spain, and Florida to fully evaluate these enantiomeric ratios of the ceralure B1 molecule.

Materials and Methods

Test Compounds. Liquid trimedlure (TML) (UOP Chemicals, East Rutherford, NJ) (98% pure; density 1.02 g/ml) was purchased from a commercial source. The enantiomers of ceralure B1, ethyl (1*R*,2*R*,3*R*)-5-iodo-2-methylcyclohexane-1-carboxylate and ethyl (1*S*,2*S*,3*S*)-5-iodo-2-methylcyclohexane-1-carboxylate, referred to as (−)-ceralure B1 and (+)-ceralure B1, respectively, based on their optical rotations, were synthesized by using a unique nine-step process (Raw and Jang 2000). Racemic ceralure was synthesized the same way with the exception of using racemic siglure acid as the starting material (Khrimian et al. 2003). Two mixtures, 87.5 and 75% (−)-ceralure B1 and racemic ceralure were tested against the pure (98%) (−)-ceralure B1 enantiomer. The 87.5% mixture was prepared by mixing a 3:1 solution of (−)-ceralure B1 and racemic ceralure and the 75% mixture was prepared by mixing a 1:1 solution of (−)-ceralure B1 and racemic ceralure. Ceralure was stored over copper coil to prevent discoloration.

Open Field Tests. All field tests were conducted by using triangular Jackson traps containing a sticky panel insert on the floor of the trap (Jang et al. 2001). Test compounds (or solvent controls) were placed on a 1.2-cm-long by 0.7-cm-diameter cotton wick in a plastic basket (AgriSense, Palo Alto, CA) and attached by wire to the inner top of the trap. Experiments were carried out in a randomized complete block design. Tests were replicated at least four times.

Wild Fly Tests. Wild fly evaluations were conducted in commercial coffee fields on the island of Kauai (Hawaii). Aliquots (100 μl) containing 10 mg of each of six treatments in acetone were applied to cotton wicks as mentioned above. Treatments were 1) commercial trimedlure, 2) 0.98% (−)-ceralure B1, 3) 87.5% (−)-ceralure B1, 4) 75% (−)-ceralure B1, 5) racemic ceralure, and 6) acetone control. Traps were hung in coffee trees in every fifth row (15 m). Within a row, traps were placed 15 m apart and 1 m above the ground. Traps were placed in six randomized blocks and serviced 7 d after being placed in the field. There were 24 replicates in four 1-wk trials.

Similar tests were conducted in Valencia, Spain. Ten milligrams of 98, 87.5, and 75% (−)-ceralure B1, racemic ceralure, TML, and acetone control were placed on a 1.2 by 0.7-cm cotton wick in Jackson traps and a sticky insert. Traps were placed in a randomized complete block design in citrus ≈10 by 10 m between traps. Traps were serviced 6 d after emplacement. There were 24 replicates in four trials.

Attraction of wild Mediterranean fruit fly to ceralure B1 also was conducted in a commercial coffee field located near Nairobi, Kenya, Africa. Ten-milligram doses of 98% (−)-ceralure B1, 75% (−)-ceralure B1, and racemic ceralure were compared with 10 mg of trimedlure and acetone control as described previously. Compounds were placed on a 1.2 by 0.7-cm cotton wick inside of a Jackson trap with a sticky insert. Six traps of each treatment were placed in rows of coffee trees spaced 2 m between rows. Traps were placed five trees apart (8 m), 1 m above the ground on every other row (5 m). Traps were serviced at 12 d after emplacement.

Sterile Fly Release Tests. Tests also were conducted with sterile released Mediterranean fruit fly in Tampa, FL. Each week 125,000 sterile adult male flies were released per square mile by air. The sterile Mediterranean fruit fly strain used was the Toliman Vienna Eight male only genetic sexing strain (Robinson et al. 1999) obtained from the USDA–APHIS mass-rearing facility in El Pino, Guatemala. In two separate tests, 100-μl aliquots containing either 20 or 40 mg each of 98, 87.5, and 75% (−)-ceralure B1, racemic ceralure, trimedlure, or acetone control were tested in Jackson traps with sticky inserts. A third test used 40 mg of 98, 75, and racemic ceralure B1 with trimedlure. Traps were tested in a randomized complete block design in citrus groves ≈15 by 12 m between traps and serviced weekly. Data for weekly trap captures were combined for analysis.

Data Analysis. Mean male trap captures were analyzed using PROC GLM followed by a Tukey's test for mean separation. A square root $(x + 0.5)$ transformation was performed on the data; however, data were presented as mean flies captured per trap per day. Significant differences were determined at the $P < 0.05$ level. The Dunnett's procedure also was performed on the data to look at pairwise comparisons of the pure (98%) (−) ceralure B1 compared with each of the other treatments. Analysis was run on SAS version 8.2 (SAS Institute 1990). Mean flies per trap per day are presented ± SEM.

Results

In tests conducted on the Island of Kauai, comparing different percentages of (−)-ceralure B1, there were no significant differences in trap captures between 98% (−)-ceralure B1, 87.5% (−)-ceralure B1, 75% (−)-ceralure B1, and racemic ceralure. But there were significant differences ($P < 0.05$) in trap captures

Table 1. Response of wild Mediterranean fruit to different percentages of (−)-ceralure B1, racemic ceralure, and trimedlure in open field tests in Kauai

Treatment (10 mg)	n	Mean flies/trap/d ± SEM
98% (−)-Ceralure B1	24	35 ± 5.1a
87.5% (−)-Ceralure B1	24	28 ± 4.5a
75% (−)-Ceralure B1	24	34 ± 5.5a
Racemic Ceralure B1	24	29 ± 4.7a
TML	24	7.6 ± 1.5b
Control	24	0.07 ± 0.03c

Data analyzed by PROC GLM; means followed by same letter in a column are not significantly different ($P < 0.05$) by Tukey's test.

Table 2. Response of wild Mediterranean fruit flies to different percentages of (−)-ceralure B1, racemic ceralure, and trimedlure in citrus orchards in Valencia, Spain

Treatment (10 mg)	n	Mean flies/trap/d ± SEM
98% (−)-Ceralure B1	24	18.3 ± 1.5a
87.5% (−)-Ceralure B1	24	14.3 ± 1.3ab
75% (−)-Ceralure B1	24	13.6 ± 1.1ab
Racemic Ceralure B1	24	13.1 ± 1.1b
TML	24	6 ± 0.63c
Control	24	0.01 ± 0.008d

Data analyzed by PROC GLM; means followed by same letter in a column are not significantly different ($P < 0.05$) by Tukey's test.

Table 3. Response of wild Mediterranean fruit flies to different percentages of (−)-ceralure B1 and racemic ceralure and trimedlure in coffee field tests in Kenya

Treatment (10 mg)	n	Mean flies/trap/d
98% (−)-CeralureB1	5	7.0 ± 0.81a
87.5% (−)-Ceralure B1	5	7.7 ± 1.2a
Racemic Ceralure B1	5	4.8 ± 0.49a
TML	5	1.0 ± 0.29b
Control	5	0.01 ± 0.01c

Data analyzed by PROC GLM; means followed by same letter in a column are not significantly different ($P < 0.05$) by Tukey's test.

Table 4. Response of released irradiated Mediterranean fruit flies to different percentages of (−)-ceralure B1, racemic ceralure, and trimedlure in Florida citrus groves

Treatment (40 mg)	n	Mean flies/trap/d ± SEM
98% (−)-Ceralure B1	6	0.64 ± 0.16a
75% (−)-Ceralure B1	6	0.77 ± 0.15a
Racemic Ceralure B1	6	0.46 ± 0.16ab
TML	6	0.11 ± 0.06b

Data analyzed by PROC GLM; means followed by same letter in a column are not significantly different ($P < 0.05$) by Tukey's test.

Table 5. Response of sterile Mediterranean fruit flies to different percentages of (−)-ceralure B1, racemic ceralure, and trimedlure in Florida citrus groves

Treatment (40 mg)	n	Mean flies/trap/d ± SEM
98% (−)-Ceralure B1	6	0.56 ± 0.2a
87.5% (−)-CeralureB1	6	0.95 ± 0.24a
75% (−)-Ceralure B1	6	0.59 ± 0.11a
Racemic Ceralure B1	6	0.38 ± 0.18ab
TML	6	0.01 ± 0.01bc
Control	6	0 ± 0c

Data analyzed by PROC GLM; means followed by same letter in a column are not significantly different ($P < 0.05$) by Tukey's test.

Table 6. Response of sterile Mediterranean fruit flies to different percentages of (−)-ceralure B1, racemic ceralure, and trimedlure in Florida citrus groves

Treatment (20 mg)	n	Mean flies/trap/d ± SEM
98% (−)-Ceralure B1	4	5.4 ± 1a
87.5% (−)-Ceralure B1	4	2.5 ± 0.42ab
75% (−)-Ceralure B1	4	1.3 ± 0.1bc
Racemic Ceralure B1	4	2.7 ± 0.85ab
TML	4	0.31 ± 0.1cd
Control	4	0.03 ± 0.03d

Data analyzed by PROC GLM; means followed by same letter in a column are not significantly different ($P < 0.05$) by Tukey's test.

between all ceralure B1 treatments compared with trimedlure and control (Table 1).

In tests conducted in Valencia, Spain, 98% (−)-ceralure B1was not significantly different from 87.5% (−)-ceralure B1 and 75% (−)-ceralure B1 but significantly different compared with, racemic ceralure B1, trimedlure, and control. 87.5% (−)-ceralure B1, 75% (−)-ceralure B1 and racemic ceralure B1 were significantly different when compared with trimedlure and control. Ceralure-baited traps captured ≈2 to 3 times more flies than trimedlure-baited traps (Table 2).

In wild fly tests conducted in Kenya, Africa, there were no significant differences between 98% (−)-ceralure B1, 87.5% (−)-ceralure B1, and racemic ceralure B1. All ceralure B1 treatments were significantly different compared with trimedlure and control (Table 3).

Results of tests conducted in Florida with sterile released Mediterranean fruit fly showed that there were no significant differences between 98% (−)-ceralure B1, 75% (−)-ceralure B1, and racemic ceralure B1. Both 98% and 75% (−)-ceralure B1 were significantly different from trimedlure. Racemic ceralure B1 was not significantly different compared with trimedlure in these tests (Table 4). In further tests conducted with sterile released Mediterranean fruit fly, by using 40 mg each of the test compounds, there were no significant differences between 98% (−)-ceralure, 87% (−)-ceralure B1, 75% (−)-ceralure B1, and racemic ceralure B1. Only 98, 87.5, and 75% (−)-ceralure B1 were significantly different compared with trimedlure and control. Racemic ceralure B1 was not significantly different compared with trimedlure (Table 5). At the 20-mg dose, the data showed that there were no significant differences between 98% (−)-ceralure, 87.5% (−)-ceralure B1, and racemic ceralure B1. (−)-Ceralure B1 (98 and 87.5%) and racemic ceralure B1 were significantly different compared with trimedlure and control (Table 6).

The data also were analyzed by pairwise comparison using a Dunnett's procedure. This analysis looked at comparisons of each of the treatments against 98% (−)-ceralure B1. In four out of six tests, the 98% (−)-ceralure B1 was not significantly different from any of the lower percentages of ceralure B1, including the racemic (±), whereas in two tests (Tables 2 and 6), the pure ceralure B1 was significantly different from the racemic ceralure B1 and the 75% (−)-ceralure B1 (analysis not shown). In every case, the pure (−)-ceralure B1 caught significantly more flies than TML.

Discussion

Previous studies have shown that (−)-ceralure B1 is a superior attractant for Mediterranean fruit fly compared with trimedlure (Jang et al. 2001, 2003); however, the cost of synthesis has been an issue with the pure enantiomer, likely to be prohibitively expensive to produce. This study was conducted to evaluate alternative formulations of (−)-ceralure B1 based on the racemic molecule, to find a more cost effective product which was equally as effective or nearly as effective as the 98% (−)-ceralure B1.

Based on the results of this study, in most locations, mixtures of (−)-ceralure B1 (87.5 and 75%) were just as effective as 98% (−)-ceralure B1 in capturing wild Mediterranean fruit flies and sterile released Mediterranean fruit flies. Racemic ceralure B1 generally captured less flies than 98% (−)-ceralure B1 but was not significantly different from pure (−)-ceralure B1 in five of six tests (Tukey's analysis). Racemic ceralure B1 caught significantly fewer flies than 98% (−)-ceralure B1 in only the tests in Spain (Table 2). This pattern was similar when we analyzed using a pairwise comparison (Dunnett's test), except for the 20-mg treatment in Florida with sterile released flies (Table 6). These results are generally similar to field tests results reported in previous articles (Jang et al. 2001, 2003) showing the racemic ceralure B1 competing favorably with the 98% (−)-ceralure B1. Ceralure B1 treatments were more attractive to wild Mediterranean fruit fly compared with trimedlure in all locations but were not significantly different from racemic ceralure B1 in two of three tests by using sterile released flies. Based on these results, we believe that racemic ceralure B1 may be a more cost-effective product for use in detection programs compared with 98% (−)-ceralure B1 given that the racemic is only slightly less attractive but likely considerably less costly to manufacture (A.K., unpublished data). Developing a (−)-ceralure B1-biased synthesis that would increase the ratio of the (−)-enatiomer to ≈75% would likely further increase the attractiveness of this molecule, approaching the effectiveness of the pure compound.

In a previous article (Jang et al. 2003), we compared both the racemic ceralure B1 and 98% (−)-ceralure B1 with the standard trimedlure (2 g) plastic plug (AgriSense) and showed that as little as 10 mg of the (−)-ceralure B1 could capture as many flies as the 2-g trimedlure plug for the first week, but trap capture was reduced after that. We also discussed that fact that based on our results "... amounts >50 mg will likely be needed to effectively compete with the commercial trimedlure plugs." Because the racemic ceralure B1 is slightly less active than the 98% (−)-ceralure B1, we would anticipate even more of the racemic ceralure B1 would be needed to compete with the 2-g trimedlure plug. Preliminary tests of racemic ceralure B1 in a polymeric matrix directly compared with the standard 2-g trimedlure plug seem to support this estimate (E.B.J., unpublished data). However, the main purpose of the current study was to evaluate the potential use of racemic ceralure B1 [or a (−)-ceralure B1-biased synthesis] as a replacement for the pure (−)-ceralure B1.

Ceralure B1 may have an important role in detection, delimitation, and control of Mediterranean fruit fly in the field. Under one scenario, racemic ceralure could replace trimedlure as the standard attractant for detection, whereas 98% (−)-ceralure B1 could be reserved for more targeted applications such as delimitation of outbreaks. Control of Mediterranean fruit fly could use either the pure or racemic B1 in combination with protein bait sprays (Dow AgroSciences, Indianapolis, IN) and/or the three-component food attractant, Biolure (Suterra Inc., Bend, OR) (which will require EPA registration if used for control).

Early detection of Mediterranean fruit fly by using pure (−)-ceralure B1 may be cost-prohibitive but less costly in the long-term if the flies could be detected early, potentially greatly reducing the costs of a subsequent eradication program. Racemic ceralure B1 is a reasonable intermediate compromise with improved attraction over TML, but without the high synthesis costs of pure (−)-ceralure B1. Further development of cost-effective synthesis of (−)-ceralure B1 will likely determine which options will be taken by state and federal action agencies. The improved attractiveness and longevity of ceralure B1 over trimedlure will be beneficial for mass trapping in areawide control if the costs of production could be reduced. A more cost-effective synthesis of racemic ceralure B1, or one that favors a greater yield of the (−)- over the (+)-enantiomer will better facilitate production of a more cost-effective commercial product with greater attractancy and longevity compared with trimedlure for use in control and detection programs.

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